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L10: Entry 31 of 37 File: USPT

DOCUMENT-IDENTIFIER: US 5646167 A
TITLE: Arylsulfonamido-substituted hydroxamix acids

Brief Summary Text (3):
Matrix-degrading metalloproteinases, such as gelatinase, stromelysin and collagenase, are involved in tissue matrix degradation (e.g. collagen collapse) and have been implicated in many pathological conditions involving abnormal connective tissue and basement membrane matrix metabolism, such as arthritis (e.g. osteoarthritis and rheumatoid arthritis), tissue ulceration (e.g. corneal, epidermal and gastric ulceration), abnormal wound healing, periodontal disease, bone disease (e.g. Paget's disease and osteoporosis), tumor growth, tumor metastasis, tumor progression or invasion, tumor angiogenesis, as well as HIV-infection (as reported in J. Leuk. Biol. 52 (2): 244-248, 1992), atherosclerosis and restenosis in angioplasty.

Brief Summary Text (5):
The compounds of the invention are inhibitors of stromelysin, gelatinase and/or collagenase activity, inhibit matrix degradation and are useful for the treatment of gelatinase, stromelysin and collagenase dependent pathological conditions in mammals, such as those cited above, including rheumatoid arthritis, osteoanhritis, tumor growth, tumor metastasis, tumor progression or invasion, tumor angiogenesis, periodontal disease, as well as the progression of HIV-infection and associated disorders, atherosclerosis, osteoporosis, and restenosis associated with angioplasty. Relevant tumors include human breast, lung, bladder, colon, ovarian and skin cancer.

Detailed Description Text (89):
The compounds of the invention exhibit valuable pharmacological properties in mammals including man and are particularly useful as inhibitors of matrix-degrading metalloproteinase enzymes (=metalloproteinases).

Detailed Description Text (93):
The above-cited properties are demonstrable in in vitro and in vivo tests, using advantageously mammals, e.g. rats, guinea pigs, dogs, rabbits, or isolated organs and tissues, as well as mammalian enzyme preparations. Said compounds can be applied in vitro in the form of solutions, e.g. preferably aqueous solutions, and in vivo either enterally or parenterally, advantageously orally, e.g. as a suspension or in aqueous solution. The dosage in vitro may range between about 10.sup.-5 molar and 10.sup.-10 molar concentrations. The dosage in vivo may range, depending on the route of administration, between about 0.1 and 50 mg/kg.

Detailed Description Text (96):
Stromelysin activity can also be determined using human aggrecan as a substrate. This assay allows the confirmation in-vitro that a compound can inhibit the action of stromelysin on its highly negatively-charged natural substrate, aggrecan (large aggregating prtoeoglycan). Within the cartilage, proteoglycan exists as an aggregate bound to hyaluronate. Human proteoglycan aggregated to hyaluronate is used as an enzyme substrate. The assay is set up in 96-well microtiter plates allowing rapid evaluation of compounds. The assay has three major steps:

Detailed Description Text (97):
1) Plates are coated with hyaluronate (human umbilical chord, 400 ug/ml), blocked with BSA (5 mg/ml), and then proteoglycan (human articular cartilage D1 -chondroitinase ABC digested, 2 mg/ml) is bound to the hyaluronate. Plates are washed between each step.

Detailed Description Text (101):
Collagenase activity is determined as follows: ninety six-well, flat-bottom microtiter plates are first coated with bovine type I collagen (35 ug/well) over a two-day period at 30.degree. C. using a humidified and then dry atmosphere; plates are rinsed, air dried for 3-4 hours, sealed with Saran wrap and stored in a refrigerator. Human recombinant fibroblast collagenase and a test compound (or buffer) are added to wells (total volume=0.1 ml) and plates are incubated for 2 hours at 35.degree. C. under humidified conditions; the amount of collagenase used per well is that causing approximately 80% of maximal digestion of collagen. The incubation media are removed from the wells, which are then rinsed with buffer, followed by water. Coomasie blue stain is added to the wells for 25 minutes, removed, and wells are again rinsed with water. Sodium dodecyl sulfate (20% in 50% dimethylformamide in water) is added to solubilize the remaining stained collagen and the optical density at 570 nM wave length is measured. The decrease in optical density due to collagenase (from that of collagen without enzyme) is compared to the decrease in optical density due to the enzyme in the presence of test compound, and percent inhibition of enzyme activity is calculated. IC.sub.50 's are determined from a range of concentrations of inhibitors (4-5 concentrations, each tested in triplicate), and K.sub.i values are calculated.

Detailed Description Text (109):
About 2 ng of recombinant truncated mouse macrophage metalloelastase (FASEB Journal Vol. 8, A151, 1994), purified by Q-Sepharose column chromatography is incubated with test compounds at the desired concentrations in the presence of 5 nM CaCl.sub.2, 400 nM NaCl, [.sup.3 H]elastin (60,000 cpm/tube), and 20 mM Tris, pH 8.0, at 37.degree. C. overnight. The samples are spun in a microfuge centrifuge at 12,000 rpm for 15 minutes. An aliquot of the supernatant is counted in a scintillation counter to quantitate degraded [.sup.3 H]elastin. IC.sub.50 's are determined from a range of concentrations of the test compounds and the percent inhibition of enzyme activity obtained.

Detailed Description Text (115):
The effect on tumor angiogenesis can be determined e.g. in rats implanted with Walker 256 carcinoma in pellets to stimulate anglogenesis from vessels of the limbus, as described by Galardy et al., Cancer Res. 54, 4715 (1994).

Detailed Description Text (116):
The effect of the compounds of the invention on atherosclerotic conditions can be evaluated using atherosclerotic plaques from cholesterol-fed rabbits which contain activated matrix metalloproteinases as described by Sukhova et al., Circulation 90, I404 (1994). The inhibitory effect of compounds of the invention, e.g. the compound of example 1(a), on matrix metalloproteinase enzyme activity in rabbit atherosclerotic plaques is determined by in situ zymography, as described by Galis et al., J. Clin. Invest. 94, 2493 (1994), and is indicative of plaque stabilization.

Detailed Description Text (156):
The present invention also relates to methods of using the compounds of the invention and their pharmaceutically acceptable salts, or pharmaceutical compositions thereof, in mammals for inhibiting the matrix-degrading metalloproteinases, e.g. stromelysin, collagenase and macrophage metalloelastase, for inhibiting tissue matrix degradation, and for the treatment of matrix-degrading metalloproteinase dependent conditions as described herein, e.g. osteoarthritis, also tumors (tumor growth, tumor metastasis, progression or invasion), pulmonary disorders, and the like described herein. Tumors (carcinomas) include human breast, lung, bladder, colon, prostate and ovarian cancer, and skin cancer, including melanoma and Kaposi's sarcoma.